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Recent Results on the Biology of Hodgkin and Reed–Sternberg cells.
II. Continuous Cell Lines

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The relative scarcity of Hodgkin (H) and Reed-Sternberg (RS) cells within biopsies from cases with Hodgkin's disease (HD) is an impediment to the analysis of the nature and function of these cells. Continuous cell lines as uniform and permanently available sources of cells provide a valid alternative. Development of HD cell lines has proven to be rather difficult when compared with the results on leukemia and Non-Hodgkin lymphoma cells. Only a few cell lines containing cells that resemble in-vivo H-RS cells have been established. Because the in-vitro culture conditions favor the self-propagation of residual normal cells, e.g. Epstein-Barr virus transformed B-lymphoblastoid cells or monocyte/macrophage monolayers, early attempts at culturing HD tissue resulted mainly in the generation of such cell lines. Even for the bona fide HD cell lines it is difficult to prove that the immortalized cells originated from an H-RS cell. These 13 HD cell lines have been extensively characterized in a large variety of aspects. These data have resulted in widely varying conclusions about the nature of the cell lines. It is apparent that all HD cell lines are unique among hematopoietic cell lines and are also different from one another. No conclusive evidence towards the origin of the cells has been obtained for some cell lines, while others could be operationally, albeit not always unequivocally, assigned to the T- or B-cell or monocyte-macrophage lineages. The overall phenotypes are often not concordant with those of normal hematopoietic cells; some cell lines show clearly mixed lineage attributes. The artifactual expansion of non-HRS cells in culture and the acquisition or loss of certain properties during the adaptation to culture systems cannot be excluded. There was also a bias for the establishment of cell lines from cases with advanced clinical stages, nodular sclerosing subtype and pleural effusions. The extensive analysis of a few cell lines has provided a wealth of information useful for the understanding of the biology of H-RS cells. The striking heterogeneity could be reflective of a biologically heterogeneous disease.

KEY WORDS: Hodgkin Reed-Sternberg cell lines

INTRODUCTION

The biological attributes and the cellular origin of the malignant cells in Hodgkin's disease (HD), the Hodgkin (H) and Reed–Sternberg (RS) cells, have been the subject of numerous investigations of fresh biopsy material in-situ[1]. These studies are hampered by the low number of H-RS cells in the neoplastic lesions and the abundance of "contaminating" normal bystander cells. Consequently, in-vitro cultivation techniques have been used in attempts to enrich and to purify H-RS cells in temporary or permanent cultures. Established cultures represent a uniform, reproducible and constantly available source of material for the characterization and identification of the tumor cells. Most of these cultures seemed to have been overgrown by fibroblasts, monocytes/macrophages and lymphoblastoid normal B-cells. Many

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Table 1 Early attempts at culturing cells from HD tissue

Cultures	*Serial passage*	*Continuous cell line*	*Cell type*	*References*
Short-term cultures	–	–	mixed morphology	15–18
Long-term cultures	+	?	polynucleated cells	19
Long-term cultures	+	–	monocytes/macrophages	20, 21
Long-term cultures	+	?	mixed morphology	22
Cell lines	+	+	EBV + LCL	23–25
Cell lines RSp + RN	+	+	EBV + LCL	26
Cell line AICHI-4	+	+	EBV + LCL	27
Cell line HD-Mar	+	+	probably T-NHL	28
Cell lines FQ, RB, SpR	+	+	non-human	29, 30

EBV + LCL = Epstein–Barr virus transformed (B-) lymphoblastoid cell lines.

continuous cell lines were dismissed as non-representative of H-RS cells[2]. Thus, development of HD cell lines has proven to be very difficult and rather fortuitous. Significant progress has been made only in the last ten to fifteen years, but only a few HD cell lines have been developed[3–14]. Most of these cell lines are still in culture and are thus available for further investigation. The purpose of the second part of this review is to present a summary of all described HD cell lines and the various characteristics of these lines.

ATTEMPTS AT CULTURING OF HD TISSUE

Numerous investigators have attempted to explore the properties of H-RS cells by means of culturing cells from involved tissue for limited periods (Table 1). Many short-term cultures contained cells of mixed morphology that usually could not be serially passaged and maintained in-vitro over a longer period[15–18]. Long-term cultures have not been clearly distinguished from lymphoblastoid B-cell lines infected with Epstein–Barr virus (EBV+ LCL). Other long-term cultures appear to have contained slow-growing monocytes/macrophages that, at some point, stopped growing[20,21].

Several continuous cell lines have erroneously been identified as having been derived from the neoplastic cell population, but are now known to represent EBV+ B-LCL[26,27]. Cultivation of HD biopsy material resulted in the establishment of EBV+ LCL in a large percentage of 149 attempts (28% and 53%) without or with the addition of exogenous EBV to the cultures[25]. In an earlier study, all of the 35 HD explants yielded lymphoblastoid lines[24]. Other cell lines have been argued to be derived from Non-Hodgkin lymphoma (NHL) or other malignant tissues[2,9,28]. Contamination of cultures by extraneous cell lines has occurred in several cell lines[29,30].

WHAT MAKES A CELL LINE A HD CELL LINE?

Because the establishment of the so-called HD cell lines was difficult to achieve in each case, it is presently nearly impossible to prove that the immortalized cell is a H-RS cell[4]. Some biological attributes have been suggested as fundamental criteria of neoplastic HD cell lines versus "normal" cell lines arising in cultures of HD tissue[20,31]: aneuploidy, heterotransplantability (tumorigenicity), and clonal derivation (Table 2). For the following reasons these criteria cannot be used as indicators of the appropriate derivation of HD cell lines. While B-LCL are initially diploid and

Table 2 Proposed attributes of neoplasia and identification of HD cell lines

Parameter	*EBV + LCL*	*HD cell lines*	*Leukemia cell lines*
Aneuploidy	(+)[1]	+	+
Monoclonality	(+)[1]	+	+
Heterotransplantability	+	+	+
Bi-/polynuclearity	(+)[1]	+	+[2]
Continuous growth in the absence of feeder layers or growth factors	+	+	+
Unlimited serial passage	+	+	+
Specific immunological marker	–	–	+[2]
Characteristic immunological marker combination	+	–	+
CD15+/CD30+	–/+	+/+	+/+[2]
Specific functional features	+	–	+
Efficiency of establishment	high	very low	low
Morphological diversity			
between cell lines	–	+	+
within cell lines	–	+	±
Clearcut assignment to a cell lineage	+	–	+

[1] After prolonged cultivation.
[2] Some cell lines are positive for this parameter.

polyclonal, with prolonged cultivation the cells become aneuploid and the pattern of immunoglobulin synthesis usually changes towards monoclonality indicating a selection in-vitro of one clone[24]. EBV+ diploid B-LCL are also capable of successful heterotransplantation into nude mice[32]. Bi- and polynuclearity cannot be regarded as a sign of neoplasia as binucleated cells are rather common in B-LCL and monocytes/macrophages, in the latter formation of polykaryons through fusion.

It is clear that no single morphological, immunological, functional or other attribute or any presently definable combination of parameters is sufficiently rigorous to permit the unambiguous identification of a giant cell in culture as having been derived from a H-RS cell[31]. In the absence of specific markers, HD cell lines could only be defined by default: while the alleged HD cell lines display one or more features associated with defined cell lineages, overall the phenotypes of all cell lines are clearly different from all hitherto described hematopoietic cell lines[6,25]. Some cell lines were described as having characteristics found on in-vivo H-RS cells[25]. However, it is difficult to agree on precisely what the characteristics and the phenotype of the H-RS cell are[1,4]. Of the numerous attempts only a dozen cell lines are regarded as "HD cell lines".

HD CELL LINES

All patients had histologically proven HD (Table 3). As no further reports were published on some of the cell lines (HuT-11, SU/RH-HD-1, ZO) it is not entirely clear whether these cultures contain immortalized cells. Unfortunately, these cell lines also remain insufficiently characterized. The cell line L-591 shows many properties consistent with a B-LCL[10] and the inclusion of this cell line in the list of HD cell lines is debatable. Another cell culture, L-439, was not a continuous cell line[9]. Modulation of the culture conditions generated the sublines L-428 KS and L-428 KSA (see below).

Two facts are striking: (i) 9/13 cell lines stem from clinical conditions similar to suspension cultures such as pleural or pericardial effusion, peripheral blood or bone marrow; thus, the adaptation to the artificial milieu clearly favors cells in effusions; (ii) 11/13 cell lines originated from patients with HD of the nodular sclerosis histological subtype. The establishment of HD cell lines is highly selective and is biased in favor of particular cells not necessarily representative of H-RS cells from other subtypes.

MORPHOLOGY

The HD cell lines show a marked heterogeneity with regard to size, form and appearance of the cells both among the various cell lines and within a culture population (Table 4). Most cells appear to be round, mono- or binucleated with a smooth cell surface or short villi, prominent nucleoli (one or more), basophilic cytoplasm that is occasionally vacuolated and a cell size of 10–30 μm. A smaller percentage of cells in each culture is bi- or multinucleated (10 or

Table 3 Cell lines derived from patients with HD

Cell line	*Histological subtype*	*Origin of material*	*Age/sex*	*Clinical stage*	*Year of establishment*	*Reference*	*Comments*
CO	nodular sclerosis	cervical lymph node	nr/F	IIIA	1981	33, 34	
DEV	nodular sclerosis	pleural effusion	51/M	II	nr	35, 36	
HD-70	nodular sclerosis	peripheral blood	69/M	II	1989	37	
HDLM-1/2/3[1]	nodular sclerosis	pleural effusion	74/M	IV	1982	38, 39	
HO	nodular sclerosis	lymph node	nr/F	II	nr	34	
HuT-11	mixed cellularity	cervical lymph node	6/F	IIA	nr	40	still alive?
KM-H2	mixed cellularity	pleural effusion	37/M	IV	1974	41	
L-428	nodular sclerosis	pleural effusion	37/F	IVB	1978	42, 43	
L-538/540[2]	nodular sclerosis	peripheral blood/bone marrow	20/F	IVB	nr	25, 44	
L-591	nodular sclerosis	pleural effusion	31/F	IVb	nr	25	EBV-lymphoblastoid cells?
SUP-HD1	nodular sclerosis	pleural effusion	37/M	IIISA	1987	45	
SU/RH-HD-1	nodular sclerosis	spleen	12/M	III	1980	46–48	still alive?
ZO[3]	nodular sclerosis	pericardial fluid	26/F	II	nr	36	continuous cell line?

[1] Three cell lines were established from the same patient.
[2] Two cell lines were established from the same patient.
[3] Dependent on IL-2; all other cell lines grow independent of exogenous growth factors.
nr = not reported.

Table 4 Morphological findings of HD cell lines

Cell line	*Cell size*	*Nucleus*	*Nucleoli*	*Cytoplasm*	*Cell surface*	*Other features*
CO	medium	large, irregular; numerous vacuoles		moderate amount of basophilic cytoplasm	smooth	lymphoid cell; occasionally large multinucleated forms
DEV	large	lobulated	prominent	small/basophilic to large/lightly stained	frequently villi	
HD-70	large	round, multiple	prominent			round cells
HDLM-2						
80%	10–20 μm	mononucleated	1–3, prominent	moderate, basophilic	smooth	occasionally vacuolated
5–10%	20–50 μm	mono-/binucleated		lighter, foamy		round or polygonal cells
10–20%	50–100 μm	polynucleated (2–10)		abundant		giant, polymorphous cell
HuT-11						
	8–14 μm	mononucleated	1 or more, prominent			round, lymphoid-like
	12–18 μm	mononucleated				polygonal
	30–48 μm	multinucleated (1–8)				giant
KM-H2		1–3, round to oval	conspicuous	pale to moderately basophilic		occasionally vacuolated
L-428						
	15–30 μm	mononucleated	large, round or kidney-shaped		smooth or "hairy" protrusions or short curbed villi	
	30–50 μm	mono-/binucleated				
	50–80 μm	multinucleated				
L-540	small/large	mono-/multinucleated	prominent	vacuolization		"bubbling cytoplasmic projections"; anisocytosis
SU/RH-HD-1						
>95%	35–60 μm	large, mononucleated	large, multiple	pale, vacuoles		round cells or forked, irregular pseudopodia
1%		binucleated				
0.1%		multinucleated				
SUP-HD1						
>80%	15–20 μm	mononucleated			smooth	round cells
15%		binucleated				
1%		multinucleated				
ZO		lobulated	huge		small, villous projections	

As published in the original descriptions; see Table 3 for references.

more nuclei), round or polygonal, large to giant of 50–100 μm or more diameter with abundant, lightly stained, foamy or vacuolated cytoplasm (Figure 1). Although some cell types were described as "lymphoid-like", no firm conclusions can be drawn to the lineage of origin from these descriptions of the morphological properties of the HD cell lines.

CYTOCHEMISTRY

Cytochemical stains of the HD cell lines showed the following staining patterns (Table 5): positivity for acid phosphatase (10/10 cell lines), positivity for α-naphthyl acetate esterase (ANAE) (10/11), positivity for Periodic Acid Schiff (PAS) (2/2), negativity for naphthol-AS-D-chloroacetate esterase (6/9), negativity for peroxidase (11/11), and negativity for alkaline phosphatase (8/8).

CELL KINETICS

The cell lines were established either in RPMI 1640 or McCoy's 5A medium supplemented with fetal calf serum (Table 6). Some cell lines started to proliferate very quickly after the initial set-up of the cultures; in others, there were long lag periods (1–2 months) before the cells finally grew permanently.

The cell lines grow either as suspension cultures with single cells or cells clustered in small or large clumps or as adherent cell cultures in monolayers. Those cell lines with "lymphoid-associated" features (e.g. immunophenotypes, gene rearrangements, etc,; see below) proliferate as free-floating cells in suspension. The cell lines with characteristics of monocytes/macrophages, HuT-11 and SU/RH-HD-1, contained adherent cells. Compared with leukemia or NHL cell lines, the HD cell lines have on average significantly longer doubling times. Due to

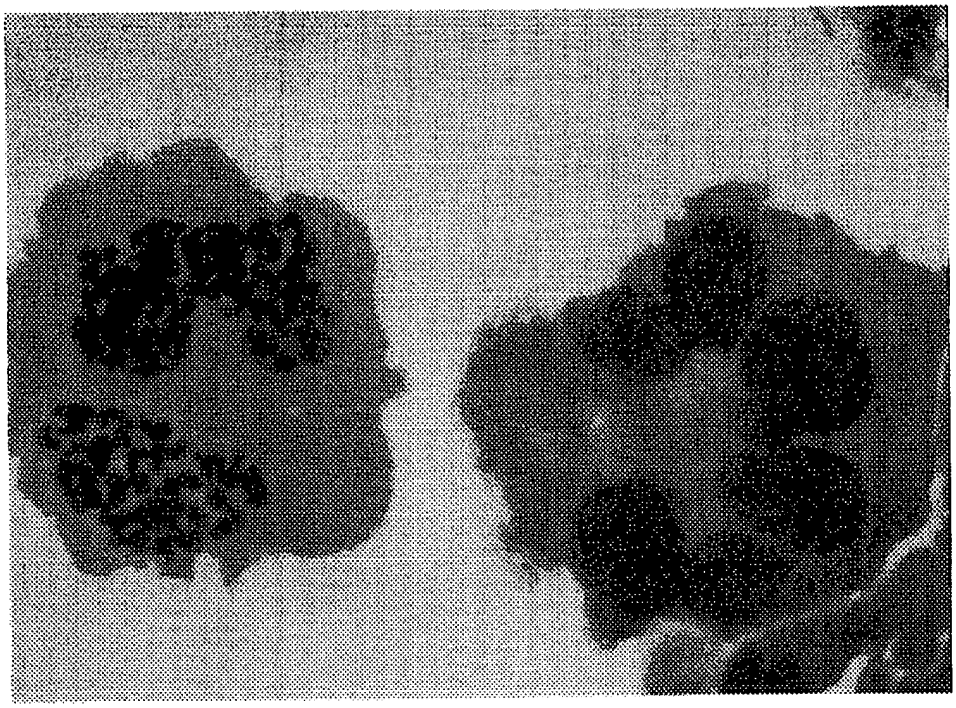

Figure 1 Two giant multinucleated HDLM-2 cells with about 50–70 μm diameter; the cell on the left shows a synchronous tri-star mitosis. May–Grünwald–Giemsa staining; magnification × 156. (See Colour Plate I at the back of this publication.)

Table 5 Cytochemical stainings of HD cell lines

Cell line	*Acid phosphatase*	*α-Naphthyl-acetate-esterase*	*Naphthol-AS-D-chloroacetate-esterase*	*Peroxidase*	*Alkaline phosphatase*	*Periodic acid schiff*	*5'-nucleotidase*	*Aminopeptidase*
CO	+	+[2]	+	−			−	−
DEV	+	−	+	−	−	+		
HD-70	+	(+)	−	−	−			
HDLM-2	+[1]	+[2]	−	−	−			
HuT-11	+	+[2]	+	−	−			
KM-H2	+[1]	+[2]	−	−	−			
L-428	+[1]	+[2]	−	−	−			
L-538/540	+	+	−	−	−			
L-591	+	+	−	−	−			
SUP-HD1	+	(+)		−		+		
SU/RH-HD-1		+		−				

[1] Inhibition by tartrate: while HDLM-2 expresses the tartrate-resistant acid phosphatase, KM-H2 and L-428 are negative.
[2] Inhibition by sodium fluoride: none of the cell lines express the monocyte-specific esterase selectivity inhibited by NaF.
See Table 3 for references.

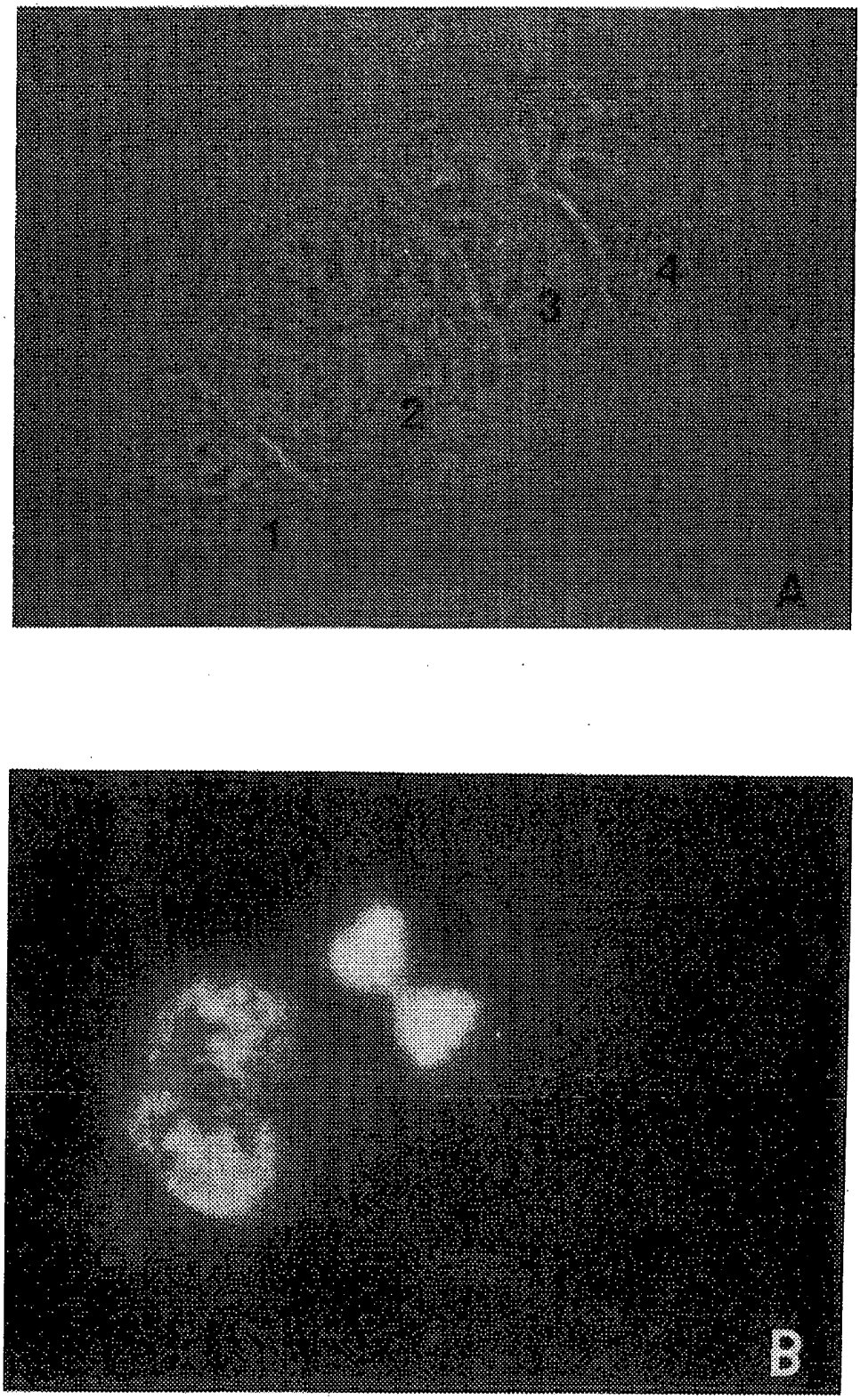

Figure 2

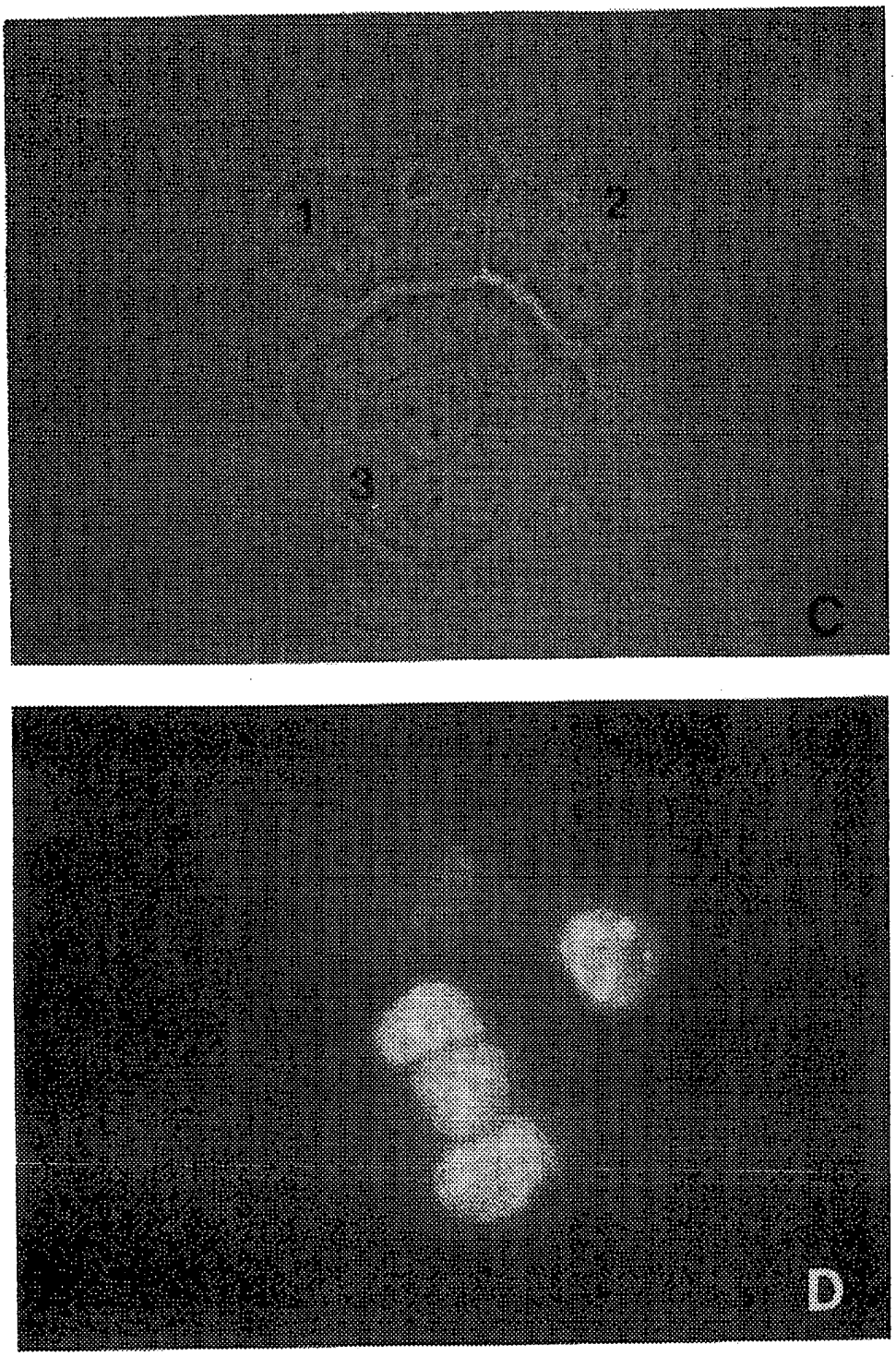

Figure 2 Immunostaining of HDLM-2 cells with Ki-67 monoclonal antibody (A/B) and anti-BrdU antibody following incorporation of bromodeoxyuridine (BrdU) (C/D). Shown are the same fields under fluorescent light and under phase contrast. (A/B) the mononucleated cells 1 and 4 are negative, the binucleated cell 2 is Ki-67 positive indicating nuclei in S, G_2 or M phase, cell 3 is positive (anaphase) (C/D) The mononucleated cell 1 is negative, the mononucleated cell 2 and the trinucleated cell 3 are BrdU positive indicating nuclei in S phase. Note that in polynucleated cells the nuclei are always simultaneously positive. Original magnification ×250. (See Colour Plate III at the back of this publication.)

Table 6. Cell kinetics of HD cell lines

Cell line	*Doubling time*	*Growth pattern*	*Initial "lag phase"*[1]	*Growth medium*[2]
CO		single cells in suspension	7 weeks	RPMI 1640
DEV	3 days	clustered cells in suspension	8 weeks	RPMI 1640
HD-70	28 hrs	single cells in suspension		RPMI 1640
HDLM-2	3–5 days	single cells in suspension		RPMI 1640
HuT-11	12 14 hrs	adherent cells as monolayers	11 weeks	McCoy's 5A
KM-H2	60 hrs	clustered cells in suspension	none	RPMI 1640
L-428	42–46 hrs	single cells in suspension	4 weeks	RPMI 1640
L-538/540		single cells in suspension	4 weeks	RPMI 1640
L-591		clustered cells in suspension		RPMI 1640
SUP-HD1	3–4 days	single cells in suspension	none	McCoy's 5A
SU/RH-HD-1	70–100 hrs	adherent cells as monolayers	1–2 months	RPMI 1640
ZO		clustered cells in suspension		RPMI 1640

[1] Initial time period between set-up and growth of cultures.
[2] Supplemented with fetal bovine serum.
As described in the original publications; see Table 3 for references.

their large size, the maximal cell densities of the HD cell lines are clearly lower than in other hematopoietic cell lines.

Using the cell lines HDLM-1/-2, KM-H2 and L-428 sufficient material was available to study the proliferative potential and the nature of the multinucleated cells by cell sorting and cloning experiments[49–51]. No difference in surface antigen expression was found between mono- and multinucleated cells[49]. After flow cytometric cell sorting or single cell cloning of mononucleated cells, polykaryons emerged subsequently in these cultures indicating that the giant multinucleated cells have arisen from the original small mononuclear cell[49,51]. Cloning was not successful with the multinuclear cells.

The observation of mitotic figures and immunostaining with the cell cycle indicators Ki-67 and bromodeoxyuridine provided evidence of DNA synthesis and synchronous nuclear division in multinucleated cells (Figure 2)[49]. However, the absence of telophases and the lack of active replication of the multinucleated cells suggested a disturbed cytokinesis. Therefore, the multinucleated giant cells appear to develop by endomitosis without cell division, always in association with mononuclear cells[49–51]. Cell fusion as a mechanism for multinucleated cell formation was not observed, but cannot be excluded entirely.

IMMUNOPHENOTYPES

Each cell line displayed a unique combination of markers (Table 7; Figure 3). Some cell lines expressed one or more markers associated with the T-cell (CO, HDLM-2, HO, L-540), B-cell (DEV, HD-70, KM-H2, L-591, SUP-HD1) or myelomonocytic cell lineages (SU/RH-HD-1). Antigens indicative of other cell lineages, e.g. erythro- and megakaryopoiesis, natural killer cells, were negative. L-428 and ZO appeared to be devoid of cell lineage-associated markers. Despite the expression of markers specific or associated with cell lineages, the overall immunophenotypes of the various lines do not correspond to any of the known stages of hematopoietic development. They are certainly incomplete; and unusual combinations of antigen expression are seen. A number of results are diverging in different reports, presumably due to clonal evolution and genetic instability of the cells and/or technical aspects. Except for the incompletely characterized SU/RH-HD-1 none of the cell lines showed a clearcut classical surface marker profile consistent with monocytes-macrophages/histiocytes.

In the studies on biopsy material a pattern of immunomarker expression by H-RS cells emerged (except for the lymphocyte predominant subtype) comprising the antigens CD15, CD25, CD30, CD71 and HLA-DR; this composite immunoprofile together with the detection of various lymphoid antigens suggests a lymphoid, activated cell[1,7]. Apart from SU/RH-HD-1 the cell lines expressed most of these antigens.

RECEPTOR GENE REARRANGEMENTS

Most of the HD cell lines were examined for their status of the specific receptors for T-cells (TCR α, β, γ, δ) and B-cells (Ig heavy and light chains): rearrangement of the genes at the DNA (Table 8),

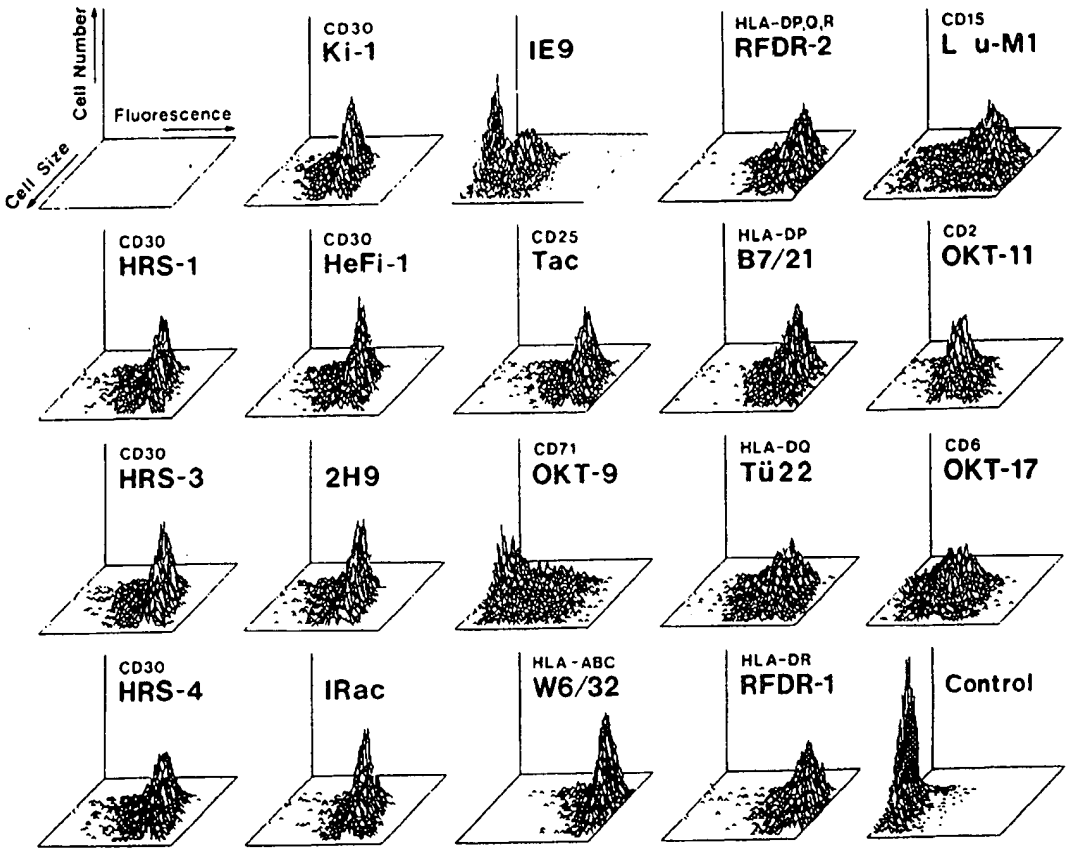

Figure 3 Flow cytometric demonstration of the surface marker immunoprofile of HDLM-2 cells. Fluorescence intensity (staining intensity on log scale) is plotted against forward-angle light scatter (cell size) and cell number. Shown are a selection of positive markers (CD groups are indicated; compare with Table 7). Small and large cells are equally positive for any given marker. Modified after Ref. 49.

transcription of the genes at the RNA (Table 8) and expression of the products at the protein level (Table 7). The results of the gene rearrangement studies are heterogeneous: 4 cell lines had TCR gene rearrangements (CO, HDLM-2, HO, L-540), 5 lines had Ig gene rearrangements (DEV, HD-70, KM-H2, L-591, ZO) and 2 lines had rearrangements of both TCR and Ig genes (L-428, SUP-HD1). Some cell lines express Ig or TCR mRNA and surface or cytoplasmic Ig or TCR proteins.

Although Ig and TCR molecules are only expressed by B- and T-cells, respectively, rearrangements of Ig and TCR genes appear not to be lineage-restricted. Thus, receptor gene rearrangements are clonality indicators rather than lineage markers. However, the gene rearrangements seen in non-lymphoid cells are commonly non-productive. The fact that some HD cell lines transcribe and translate the Ig or TCR genes may point to an immature lymphoid origin of these cells[5].

CYTOGENETICS

A specific chromosomal marker of H-RS cells has not yet been defined[1]. While there are enough mitoses and no contaminating normal cells, the karyotyping of HD cell lines is a very difficult exercise as the karyotypes of these cells are highly complex and also unstable over extended periods of culture. All cell lines are aneuploid, most of them hyperdiploid (Table 9). Surprisingly, the HDLM-2 cells are hypodiploid with only 36 chromosomes, but they carry a host of abnormalities. All cell lines so far analyzed display numerous structural abnormalities. Various cellular oncogenes, e.g. bcl-1, c-ets 1, c-ets 2, c-myb, c-myc,

Table 7 Immunophenotypes of HD cell lines

Marker (specificity) CD	*CO*	*DEV*	*HD-70*	*HDLM-2*	*HO*	*KM-H2*	*L-428*	*L-540*	*L-591*	*SU/RH-HD-1*	*SUP-HD1*	*ZO*
T-cell associated:												
CD1a thymus	–	–	–	–	–	–	–					
CD2 pan-T (LFA-3 R)	–	–	–	+	–	–	–	±?	+		–	–
CD3 pan-T (TCR-associated)	cy+	–	–	–	+	–	–	–	–		–	–
CD4 T-helper/inducer	–	–	–	±?	+	±?	–	+	–	–	–	cy+
CD5 pan-T	±?	–	–	–	+	–	–	–	–	–	–	–
CD6 pan-T				–		–	–					
CD7 pan-T	+	–		–	+	–	–	–	–		–	–
CD8 T-suppressor/cytotoxic	–	–	–	–	–	–	–	–	–	–	–	–
— TCR α/β	–		–	–	±?	–	–	–	–		–	
— TCR δ				–		–						
B-cell associated:												
CD9 immature B-cells				–		+	–					
CD10 cALL antigen	–	–		–		–	–	–	–			–
CD19 pan-B	–		–	–	–	–	±?	–	+		–	–
CD20 pan-B	–	+	–	–		–	–	–	+		–	–
CD21 pan-B (C3d R)	–	–	–	–		±?	–				–	–
CD22 pan-B	–	+		–	–	±?	–	–	±?		–	–
CD23 B-cells (FcE RII)	–				–	–	–	+	–			
CD24 B-cells	–		–	–		–	±?					
CD37 B-cells		–									–	–
CDw/75 mature B-cells							–					
— Ig heavy/light chains	–	cyIgA	cyIgAκ	–	–	–	–	–	sIgAλ	–	sκ	–
— FMC-7 (mature B-cells)				–		–	–					
Myeloid/monocytic cell-associated:												
CD11b myelomonocytic (C3Bi R)	–	–		–		–	–	±?	±?			
CD11c myelomonocytic	–	–		–	–	–	±?	–	+		–	–
CD13 pan-myelomonocytic	–		–	–	–	–	–					
CD14 monocytic	–		–	–		–	±?	+	(+)	+	–	
CD33 pan-myelomonocytic	–		–	–	–	–	–					
CD36 myeloid/megakaryocytic			–									
CDw65 pan-myelomonocytic							(+)					
CD68 monocytic/macrophages				–		–					–	
— myeloperoxidase							–					
Erythroid cell-associated:												
— glycophorin A				–		–	–					
Megakaryocytic cell-associated:												
CD41 platelet gp IIb/IIIa complex							–					
CD42b platelet gp Ib							–					
Natural killer cell-associated:												
CD16 natural killer cells (FcR III)	–			–		–	–				–	
CD56 natural killer cells				–		–	–				–	–
CD57 natural killer cells	–	–	–	–		–	–			–	–	–
Leukocyte adherence molecules:												
CD11a LFA-1α				–		–	–					
CD18 LFA-1β				–		(+)	–					
CD54 ICAM-1				+		+	+					
CD58 LFA-3				+		+	+					
HD-associated:												
CD15 myeloid/HD-associated	+	+	+	+	–	+	+	(+)	±?	–	+	+
CD30 activation/HD-associated	+	+	+	+	+	+	+	+	+	–	–	+
CDw70 activation/HD-associated	–				+	+	+	+	+			

(continued)

Table 7 (*continued*)

Marker (specificity) CD	*CO*	*DEV*	*HD-70*	*HDLM-2*	*HO*	*KM-H2*	*L-428*	*L-540*	*L-591*	*SU/RH-HD-1*	*SUP-HD1*	*ZO*
Others:												
CD25 interleukin-2 R	−	+	−	+	−	+	±?	+	+		+	+
CD34 precursor cells				−		−	−					
CD38 activation antigen	−		−	−	(+)		−					
CD43 leukosialin		−										−
CD45 common leukocyte antigen	+	+	−			−	−				−	−
CD71 transferrin R	+	+	+	+	+	+	+				+	
CD74 class II-associated											+	
— R4/23 (dendritic reticulum cells)	−					−	−	−				
— terminal deoxynucleotidyl transferase	−			−		−	−	−	−		−	
— HLA-A, B, C class I				+		+	−			+		+
— HLA-DR class II	±?	−	−	+	+	+	+	+	+	+	+	+

(+) = weak expression or only on a subpopulation, ±? = diverging results reported in different publications; R = receptor; TCR = T-cell receptor; Ig = immunoglobulin; cy = cytoplasmatic expression; s = surface membrane expression; CD groups according to the four Workshops on Human Leukocyte Differentiation Antigens.
References: 25, 33–39, 41, 44–46, 51–61.

met, N-ras and others, are localized in the chromosome regions involved in these abnormalities[5].

The inherent cytogenetic instability of the cell lines is exemplified by the different karyotypes published over a period of about 10 years for the best-cell line, L-428[5,25,44,51,67,68]. For the interpretation of chromosomal aberrations in the HD cell lines one has to keep in mind that all lines were derived from patients who underwent radiotherapy and/or chemotherapy with mutagenic agents[9].

ONCOGENES

The pattern of expression of proto-oncogenes is heterogenous among the cell lines (Table 10); no HD-associated or -specific combinations or alterations could be detected. Aberrant transcripts of fes that are not found in untransformed hematopoietic cells were seen in the cell lines CO and L-428[63]. Weak expression of the c-fos and c-jun proteins was described for HDLM-1 and KM-H2[70]. Regarding mutations of the N-, Ki- and H-ras oncogenes, only

Table 8 Gene rearrangement status of HD cell lines

Gene	*CO*	*DEV*	*HD-70*	*HDLM-2*	*HO*	*KM-H2*	*L-428*	*L-540*	*L-591*	*SUP-HD1*	*ZO*
T-cell receptor:											
alpha chain	G−	G		R			G+	R+			G
beta chain	R+	G	G	R	R+	G	R?−	R−	G	R	G
gamma chain	R−		G	R	R	G	G−	R−	G		G
delta chain			G	D		G	G	D			
Immunoglobulin:											
heavy chain											
J_H	G−	R	R	G	G−	R−	R+	G	R	R	R
Cα							G		+		
Cγ	G−						R±?	G−	D−		
Cε							G				
Cμ	G−						D−	G−	D−		
light chain											
kappa	G−	R	R	G	G	R?	R?−	G−	G+	R+	R
lambda	G−			G	G	G	R?−	G−	R+	G	G

R = rearrangement; G = germline configuration; D = deletion; R? = diverging results in different reports. + = expression of the receptor gene at the RNA level (for the production of the proteins see Table 7); ±? = different results in the literature.
References: 10, 34, 36, 37, 41, 45, 52, 54–57, 61, 63–65.

Table 9 Cytogenetic abnormalities of HD cell lines

Cell line	*Chromosome no.*	*Localizations of non-random chromosome aberrations*	*References*
CO	86–90	8, 9p, 18, 19, 20, Xp	33
DEV	48	t(3;14), t(3;22), t(3;7), del(3)	35
HD-70	74	1q13, 6p21, 7q22, 8q24, 11p11–13, 12p13, 13qcen, 14q32, 17p13	37
HDLM-2	36	1p32, 3q13, 3q27, 6q23, 7p14, 9p11, 9p12, 11p11, 12q24, 19p13, 22q11	66
HuT-11	37 190		40
KM-H2	44 – > 100	2q, 4q, 5p, 6p, 7q, 10p, 14p	41
L-428	48–50	1p22, 2p25, 2q33, 6q23, 7q22, 9p24, 11q21, 12q15, 13p12, 14q32, 21q21	25, 44, 51, 67, 68
L-538/540	66–71	1p22, 2q33, 5q15, 6p25, 8q24.1, 11q21, 11q23, 12q22, 15p12, 21p12	25, 44, 67–69
L-591	46, 92	7q32–7q36, 14q32.1	25, 67, 68
SUP-HD1	44	1p13, 1q32, 1q44, 2p23–25, 2p25, 4q31, 5p15.3, 7p15, 8p21–23, 8p22 11p15, 11q23–25, 14p11.2–q11.2, 21q21–22.3, 22q13	45
SU/RH-HD-1	44–47		46
ZO	53	iso1q, t(1;13), iso2p, 4q-, 6q-, t(7;17), 16p+, 16q–	36

cell line CO contained a mutated Ki-ras gene[71]. The significance of these findings remains to be elucidated. Possibly the differences in proto-oncogene expression are related to various stages of differentiation or activation or might be caused by tumor-induced deregulation[5].

CYTOKINES

The humoral interaction of HD cells with immunocompetent cells via cytokines appears to be of great importance in the pathophysiology of the disease. Some aspects of the biology and clinical presentation of HD suggest the involvement of cytokines[5].

HD cell lines express a variety of cytokine genes either constitutively or upon activation with different inducers (Table 11): colony-stimulating-factors (GM-CSF, M-CSF), interferons (IFN-γ), interleukins (IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9), tumor necrosis factors (TNF-α and TNF-β) and others. The receptors for some cytokines were found on the cells as well: IL-2 R, IL-4 R, IL-6 R, IL-9 R, M-CSF R, TNF-α R (Table 11).

These observations raised the question as to whether the cells are dependent upon growth factors or can be stimulated by the cytokines in an autocrine or paracrine fashion. However, apart from some exceptions (IL-4, IL-9) most of the cytokines do not increase the growth of the cells nor does inhibition of the cytokines cause any reproducible effects (Table 12) suggesting that the cell lines contain, indeed, autonomously growing, cytokine-independent cells.

Recently, the gene encoding the CD30 antigen has been cloned[89]. The CD30 antigen shares common features with tumor necrosis factor receptors and with nerve growth factor receptor. These results indicate that the CD30 antigen is the receptor for one or more yet unidentified growth factors. CD30 might be responsible for cytokine-directed growth regulation.

FUNCTIONAL FEATURES

Besides the functional characteristics of HD cell lines mentioned above a large variety of other functional properties have been tested (Table 13). Unfortunately, not all cell lines were analyzed with the same methods and some cell lines were not analyzed at all.

Epstein–Barr Virus: With the exception of L-591, none of the HD cell lines had been transformed by Epstein–Barr virus (EBV). These tests included assays for the detection of EBV-associated nuclear antigen (EBNA), viral capsid antigen (VCA), early antigen (EA) and EBV-specific DNA sequences in the genome of the cell lines. The EBV-receptor was also found on L-428 cells, but they do not carry EBV genes[25]. According to strict criteria for the definition of HD cell lines, L-591 might not qualify as a cell line containing H-RS cells. It was therefore argued that L- 591 might be derived from an EBV-immortalized lymphocyte from within the HD's lesion and is thus unlikely to be related to the HD tumor cell population[93,100].

Heterotransplantation: Xenogeneic growth in nude mice is a property considered to be indicative of malignant potential[31]. Tumorigenicity of variable degree and latency was found for most, but not all HD cell lines for subcutaneous, intraperitoneal or intracranial transplantation into nude (SCID) mice.

Table 10 Studies on oncogene expression and mutations in HD cell lines

Cell line	*Nuclear oncogenes*							*Protein kinases*									*Different functions*				*Point mutations*		
	c-myc	*N-myc*	*L-myc*	*myb*	*p53*	*fos*	*jun*	*src*	*fgr*	*syn*	*lck*	*fes*	*met*	*pim*	*raf*	*mos*	*sis*	*mas*	*dbl*	*N-ras*	*Ki-ras*	*H-ras*	*N-ras*
CO	+	(+)	–	+	+	–	(+)	–	–	+	+	+[a]	–	–	+	–	–	–	–	+	+[p]	–	–
DEV	+	–		(+)	–	–	(+)	–	–		–	–	–	+	+		–			+			
HDLM-1						(+)[i]	(+)[i]																
HDLM-2						+	+														–	–	–
KM-H2						+	+														–	–	–
L-428	+	–	–	(+)	+	–	+	–	–	+	–	+[a]	+	+	+	–	–	–	–	+	–	–	–
L-540	+	–	–	(+)	+	–	+	–	–	–	–	–	+	+	+	–	–	–	–	+	–	–	–

+ = mRNA expression; (+) = weak expression; +[a] = aberrant transcripts; +[p] = point mutation; – = not detected; [i]expression of the protein in immunostaining.
References: 56, 63, 65, 70, 71.

Table 11 Expression of cytokines and cytokine receptors in HD cell lines

Cytokine (receptor)	*Level*	*CO*	*DEV*	*HDLM-1/-2*	*KM-H2*	*L-428*	*L-540*	*L-591*	*SUP-HD1*
G-CSF	RNA	−		−	−	−	−	−	
	Protein	−	−	−	−	−[1,3]	−[1]	−[1]	
GM-CSF	RNA	−		+[2]	+[2]	+	+	+	
	Protein			+[2]	+[2]				
IFN-γ	RNA			−	−				+
	Protein			−	−				+[2]
IL-1α	RNA			+	±?				
	Protein	−	−	±?	+[2]	−[1]	−[1]	+	
IL-1β	RNA	−		−	±?	−	−	−	
	Protein	−	−	−	−	−	−	−	
IL-2	RNA	−		−	−	−	−	−	−
	Protein	+[2]	−	−	−	−	−	−	
IL-3	RNA			+[2]	+[2]				
	Protein	−	−	+[2]	−	−	±?	±?	
IL-4	RNA	−		±?	−	±?	−	−	
	Protein	−	−	±?	−	+	−	−	
IL-5	RNA	−		±?	±?	+	−	−	
IL-6	RNA	−		+	±?	+	−	+	
	Protein	−	−	+	+	+	−	+	+
IL-7	RNA			−	−				
IL-8	RNA			+[2]	+[2]				
	Protein	+[2]	+	+[2]	+[2]	−	−	+	
IL-9	RNA			+	+				
	Protein			+	+				
IL-10	RNA			−	−				
LIF	RNA			+	+[2]				
M-CSF	RNA			+	+	+			
	Protein			+	+	+[3]			
TGF-β	RNA			+	+	+			
	Protein			−	−	+			
TNF-α	RNA	+		+	+	+	+	+	
	Protein	−	−	+	+	+	±?	+	+
TNF-β	RNA			+	+	+	+		
	Protein	−	−	+	+	+	+	+	
IL-2 R (CD25)	RNA	±?		+/+[4]	+/+[4]	+	+	+	+
	Protein	−	+	+/±?[4]	+/−[4]	±?	+	+	+
IL-4R	Protein					+			
IL-6 R	RNA	+		+	−	+	+	+	
	Protein	+	−	+	±?	−	+	+	+
IL-9 R	Protein			+	+				
M-CSF R (c-fms)	RNA			+	+	+			
	Protein			+	+	+[3]			
TGF-β R	Protein					−			
TNF-α R	Protein			+	+				+

[1] Described as positive in earlier reports.
[2] Only positive after treatment with phorbol ester TPA.
[3] The sublines L-428 KS and L-428 KSA are positive.
[4] IL-2 receptor p55/p75.
±? = Diverging results in different reports; mRNA expression was detected by Northern blotting analysis; protein production was examined by immunostaining, bioassays (ELISA or indicator cell line) or Western blotting. Abbreviations: CSF = colony-stimulating factor; G-CSF = granulocyte-CSF; GM-CSF = granulocyte/macrophage-CSF; IFN = interferon; IL = interleukin; LIF = leukemia inhibitory factor; M-CSF = macrophage-CSF; R = receptor; TGF = transforming growth factor; TNF = tumor necrosis factor.
References: 45, 51, 53, 56, 72 88.

Lysozyme Production and NBT Reduction: The monocyte/macrophage property of lysozyme production could not be demonstrated in any of the cell lines analyzed. Neither HDLM-2 nor L-428 reduced nitroblue tetrazolium (NBT), indicative for normal granulocytes and monocytes.

Phagocytosis: Except for HuT-11, L-428 KSA and SU/RH-HD-1, the cell lines do not display phagocytosis of coated erythrocytes, latex beads or iron particles; phagocytosis is an attribute of monocytes-macrophages.

Stimulation of MLR, Accessory Cell Function, Antigen Presentation: L-428 cells were found to be potent stimulators of the primary human mixed lymphocyte

Table 12 Effects of cytokines and cytokine inhibitors on growth of HD cell lines

Cytokine/cytokine receptor	*HDLM-1/-2*	*KM-H2*	*L-428*	*L-540*	*L-591*
Effects of cytokines					
G-CSF	–	–			
GM-CSF	–	–			
IFN-α	–	–			
IFN-β	–	–			
IFN-γ	–	–			
IL-1	–	–			
IL-2	–	–	–	–	
IL-3	–	–			
IL-4			S		
IL-6	–				
IL-9	–	S			
M-CSF	–	–	I		
TGF-β			–		
TNF-α	–	–			
Effects of anti-cytokine Ab:					
anti-IL-2 Ab	–	–			
anti-IL-4 Ab			–		
anti-IL-6 Ab	–	–			–
anti-IL-9 Ab	I	–			
anti-M-CSF Ab	–	–	–		
anti-TNF-β Ab	–				
Effects of anti-cytokine receptor Ab:					
anti-IL-2 R Ab	–	–			
Effects of anti-sense RNA:					
anti-sense IL-9RNA	I				

[1] Subline L-428 KSA is inhibited.
– = No stimulatory or inhibitory effect; Ab = antibodies; S = stimulation; I = inhibition.
Abbreviations: see Table 11; References: see Table 11.

reaction (MLR)[90]. L-428 cells also function as accessory cells for mitogen-induced, human T-cell proliferative responses[91]. These latter two findings led to the suggestion that L-428 cells are related to dendritic cells[90,91]. L-428 and SU/RH-HD-1 are capable of presenting soluble antigen to T-cells in a genetically restricted fashion[46,92].

Spontaneous Rosetting of T-Cells: Spontaneous rosetting of T-lymphocytes to H-RS cells has been observed both in-vitro and in-vivo. This rosetting is mediated by interaction of T-cell CD2 with its ligand LFA-3 (CD58) on the H-RS cells. A second mode of binding occurs via LFA-1 (CD11a/CD18) on T-cells to ICAM-1 (CD54) on L-428 cells[60].

Rosette-Inhibiting Factor, Neutrophil-Migration Inhibitor, Fibroblast-Activating Factor: L-428 cells secrete a factor, termed rosette-inhibiting factor (RIF) that suppresses the binding of sheep red blood cells to normal peripheral blood T-lymphocytes (E rosetting)[94]. Another factor secreted by L-428 and its subline L-428 KSA inhibits both random migration and migration towards chemo-attractants of normal blood neutrophils[95]. An activity was demonstrated in the supernatant of L-428 that stimulates fibroblast proliferation[61]. These three factors remain only partially characterized and might as well be related to some of the cytokines described above.

Glycoprotein Expression: The expression of a 55 kD protein, variously termed "HD-protein" or "HD-lectin", was described for the cell line L-428[96]. This ectosialytransferase functions as carrier protein for the X-hapten oligosaccharide and was also detected on non-HD cell lines[97]. The surface-expressed "HD-lectin" and its soluble counterpart are lymphocyte adhesion molecules (distinct from other accessory molecules) and mitogen[99]. The relationship of this protein to the well-known leukocyte adherence antigens remains to be established. Other peanut-agglutinin binding proteins appeared to be restricted to HD cell lines when compared with non-HD cell lines; galactosylation processes were apparently aberrant in these cells[100].

Table 13 Functional studies on HD cell lines

Feature	*Positive (or examined)*	*Negative*	*References*
Transformation by EBV	L-591	CO, DEV, HD-70, HDLM-2, HO, HuT-11, KM-H2, L-428, L-540, SUP-HD1, SU/RH-HD-1, ZO	33–38, 40, 41, 44–46, 53, 57, 65
Heterotransplantation	DEV, HD-70, HDLM-2, HuT-11, L-428, L-540, L-591, ZO	KM-H2, SUP-HD1, SU/RH-HD-1	25, 35–37, 40, 41, 44–46, 53, 61
Lysozyme production		CO, DEV, HuT-11, KM-H2, L-428, L-540, L-591, SU/RH-HD-1	25, 33, 35, 40, 41, 44, 46
NBT reduction		HDLM-2, L-428	38
Phagocytosis	HuT-11, L-428 KSA[1], SU/RH-HD-1	CO, DEV, HDLM-2[2], KM-H2[2], L-428, L-540, L-591	25, 33, 35, 38, 40, 41, 44, 46
Stimulation of MLR	L-428		90
Accessory cell function	L-428		91
Antigen presentation	L-428, SU/RH-HD-1	HDLM-2	39, 46, 92
Spontaneous rosetting of T-cells	CO, L-428, L-591	L-540	25, 33, 44, 59, 60, 93
Rosette-Inhibiting Factor (RIF)	L-428		94
Neutrophil-migration inhibition	L-428, L-428 KSA[1], L-540		95
Fibroblast-Activating Factor	L-428		61
Glycoprotein expression	CO, HO, L-428, L-428 KS[1], L-428 KSA[1], L-591		96–100
Natural killer (NK) activity		HDLM-2	39
Isoenzyme studies	CO, HDLM-2, HO, KM-H2, L-428 L-540, L-591		33, 38, 101
Nuclear transcription factors	NFAT-1: HDLM-2, KM-H2 NFκB: HDLM-2, KM-H2, SUP-HD1 AP-1: HDLM-2, KM-H2	NFAT-1: SUP-HD1	45, 56
Prostaglandin synthesis	HDLM-1, KM-H2		58,102

[1] Sublines of L-428.
[2] A subpopulation of HDLM-1 and KM-H2 could be induced by TPA to phagocytose yeast particles.
Abbreviations: MLR = mixed lymphocyte reaction; NBT = nitroblue tetrazolium.

Natural Killer Activity: Only the cell line HDLM-2 was tested for natural killer (NK) activity and found to be negative[39]. None of the cell lines expresses surface markers associated with NK cells (CD16, CD56, CD57) (see Table 7).

Isoenzyme Studies: Cellular α-naphthyl acetate esterases, acid phosphatases, hexosaminidases and dipeptidylpeptidase IV were analyzed quantitatively and qualitatively[101]. The results indicate that all of the cell lines examined had enzymatic features typical for lymphoid cells. In particular, a monocyte/histiocyte-associated isoenzyme pattern could be excluded.

Nuclear Transcription Factors: Nuclear extracts were assayed for the expression of proteins known to be transcription factors that bind to DNA sequences of many cytokine genes: Activation Protein (AP) 1, Nuclear Factor of Activated T-cells (NFAT-1; previously demonstrated to be T-cell specific), and Nuclear Factor kappa B (NFκB; associated with B-cells). The constitutive activity of all three factors could be further augmented by TPA[56].

Prostaglandin Synthesis: The cell line KM-H2 was found to produce large amounts of prostaglandin E_2, a property of histiocytes and monocytes, while HDLM-1 synthesizes only small quantities[58]. The extent of prostaglandin production and secretion correlated with the presence of the enzyme prostaglandin H synthase (cyclooxygenase) required for the synthesis of prostaglandin[102].

SUBLINES

Sublines of HDLM-1 and L-428 were derived by modulation of the culture conditions (Table 14). HDLM-1, -2 and -3 were set up as independent cultures from the same patient and material (pleural effusion). L-538 and L-540 are two cell lines from the same patient, but one was established from a peripheral blood sample and the other from a bone marrow specimen, respectively (Table 3). The suspension cell line L-428 KS is a variant showing features similar or identical to those of the parental L-428[25].

Table 14 Sublines of HD cell lines

Subline	*Parental cell line*	*Mode of establishment*	*Growth pattern*	*Features*	*References*
HDLM-1d	HDLM-1	treatment with TPA	in clumps in suspension (in TPA-free medium)	mostly similar, some features different from HDLM-1	50, 59, 75, 78, 103
L-428 KS	L-428	adaptation to calf serum	single cells in suspension	similar to L-428	25, 61, 104
L-428 KSA	L-428	treatment with TPA	adherent monolayer (in TPA-free medium)	some properties of monocytes-macrophages	25, 61, 104

The L-428 KS cells exhibit a marked chromosomal instability with several additional cytogenetic markers. Exposure of the L-428 KS cell line to the phorbol ester TPA resulted in the new subline L-428 KSA[25]. This cell line subsequently maintained in the absence of TPA grows as an adherent monolayer culture with features of epitheloid cells and properties of monocytes-macrophages[61,104].

The sublines HDLM-1d and HDLM-2d were cultured in the presence of TPA and subsequently in TPA-free medium[50]. The HDLM-1d cells have many similar, but also some different characteristics when compared with HDLM-1[50,59,75,78,103] for instance, contrary to the growth of HDLM-1 as single cells, HDLM-1d proliferate in clumps.

INDUCTION OF DIFFERENTIATION

Many physiological and pharmacological substances known to induce the differentiation of leukemia and lymphoma cells have become available. It is commonly assumed that the malignant cells that are arrested in their maturation will be induced by the biomodulators to differentiate further along their predetermined cell lineage pathway. Such differentiation can be coupled with the appearance of new markers indicating the cell lineage.

Several HD cell lines have been exposed to differentiation inducing agents, e.g. the phorbol ester TPA, the vitamin A-analogue retinoic acid and to extracellular matrix (Table 15). Treatment with these

Table 15 Studies on induction of differentiation of HD cell lines

Cell line	*Treatment*[1]	*Induced effect*	*Induced new expression*	*Increased expression*	*References*
CO	TPA		CD3, TCR α/β IL-2, IL-8	CD25	105
HDLM-1	TPA	morphological changes (changes reversible), increase of polynucleated cells, block at G_0/G_1 exit into S phase		IL-1, TNF-α, TNF-β	50, 75, 76, 103
HDLM-1	ECM	adherence, morphological changes			103
HDLM-1	TPA + RA + ECM	adherence, morphology of histiocytes, phagocytosis (subpopulation)	CD11c, CD13, CD14, CD33, CD68, loss of CD30	IL-1, TNF-α, M-CSF, esterase, acid phosphatase, lysozyme	58
HDLM-2	TPA		cytokines (see Table 11)	AP-1[2], NFκB[2], NFAT-1[2]	56, 74
HDLM-2	TPA	stop of proliferation		esterase and acid phosphatase isoenzymes	38
KM-H2	TPA + RA + ECM	adherence, morphology of histiocytes, phagocytosis (subpopulation)	CD11b, CD11c, CD13, CD14, CD25, CD68, loss of CD30	IL-1, TNF-α, M-CSF, esterase, acid phosphatase, prostaglandin E_2	58, 75, 76
KM-H2	TPA		CD33, CD34, cytokines (Table 11)	AP-1[2], NFκB[2], NFAT-1[2]	56, 74
L-428	TPA	stop of proliferation		esterase and acid phosphatase isoenzymes	38 38
SUP-HD1	TPA		IFN-γ		45

[1] ECM = extracellular matrix; RA = retinoic acid; TPA = phorbol ester 12-0-tetradecanoylphorbol 13-acetate.
[2] Nuclear transcription factors.

inducers caused a growth arrest paralleled by distinct morphological changes and new or increased expression of various features. The single most potent agent is TPA; retinoic acid or cytokines alone do not appear to be effective[77]. A maximum degree of differentiation of the cells could be achieved with a combination of TPA, extracellular matrix and retinoic acid[58]. Following exposure to these inducers the cells displayed features of histiocyte-like cells, e.g. morphology, phagocytosis, surface marker expression, cytochemical stainings, etc.[58] Based on the assumption that differentiation studies are more specific than evidence obtained from phenotypic analysis alone, these results were viewed as indicative of an origin of H-RS cells from cells of the histiocyte lineage[58].

However, immature cells or cells arrested at bifurcation points can be induced to differentiate along various cell lineages: using various inducers the myeloid HL-60 cells acquire properties of monocyte/macrophage-type cells, neutrophils or basophils; the pre B-ALL cell line REH can be triggered by TPA to show attributes of macrophages; depending on the conditions applied erythroleukemic K-562 cells have been reported to differentiate along erythroid, megakaryocytic, myeloid or monocytic lineages.

HDLM-2 cells produce a factor(s) that could induce differentiation in several leukemia cell lines[38]. These at the time unidentified differentiation-inducing growth factors do certainly belong to the set of cytokines now known to be secreted by HDLM-2 cells (Table 11). Apparently the substance responsible for the differentiation of the treated cells is TNF and not IFN-γ as the differentiation effect can be neutralized with monoclonal anti-TNF antibodies[76].

MONOCLONAL ANTIBODIES

Attempts were made to raise monoclonal antibodies (McAbs) against antigens on HD cell lines (Table 16). The first McAb Ki-1, initially thought to be specific for H-RS cells[109], stains normal T-cells, B-cells and monocytes/macrophages following activation with various stimuli[108,113]. The antigen was assigned to cluster CD30 ("H-RS cell associated", "activation antigen") and the gene encoding the antigen has recently been cloned[89]. Other CD30 McAbs were raised against L-428 (HRS-1, HRS-2, HRS-3, HRS-4, HeFi-1) and against CO (the formol-resistant Ber-H2)[106–108]. The new McAb BLA.36 against HDLM-3 remains only partially characterized[111]. Ki-24 detects the CDw70 antigen ("activated T- and B-cells, H-RS cells"). Ki-67 reacts with a nuclear antigen that is associated with cellular proliferation and that is expressed only in late G_1, S, G_2, and M phase[112].

CONCLUSIONS

The fragility of freshly isolated H-RS cells and the contamination with numerous reactive cells have hampered the short-term analysis and attempts to establish long-term cultures from biopsy material[44]. The continuous cell lines, in long-term cultivation and propagation of the cells >1 year, provide the only likely alternative for studying several phenomena of HD in detail[93]. The difficulty of growing neoplastic cells from HD tissue in-vitro is indicated by the rarity of established HD cell lines in comparison with leukemia and NHL cell lines[83]. Regardless of all variations applied in the set-up of the cultures, the in-vitro system favors the outgrowth of lymphoblastoid cells and macrophage-type cells and seems to be suboptimal for the proliferation of truly neoplastic cells[42]. In retrospect, prior to 1978 the cells cultured from HD biopsies were probably, for the most part, EBV+ lymphoblastoid cell lines (LCL).

In the last 15 years cell lines (Table 17) have been derived that, on the basis of a panel of identical or

Table 16 Monoclonal antibodies against HD cell lines

Monoclonal antibody	*Cell line*	*CD group*[1]	*Reactivity pattern*[2]	*References*
Ber-H2	CO	CD30	H-RS cells, activated T- and B-cells, monocytes	106
HeFi-1	L-428	CD30	H-RS cells, activated T- and B-cells, monocytes	107
HRS-1/-2/-3/-4	L-428	CD30	H-RS cells, activated T- and B-cells, monocytes	108
Ki-1	L-428	CD30	H-RS cells, activated T- and B-cells, monocytes	109
Ki-24	L-428	CDw70	H-RS cells, activated T- and B-cells	110
BLA.36	HDLM-3	—	H-RS cells, B-NHL as, subset of normal B-cells	111
Ki-67	L-428	—	expressed in late G_1, S, G_2, M phase	112

[1] CD = cluster of differentiation according to the Third and Fourth Workshops on Human Leukocyte Differentiation Antigens.
[2] Ki-67 is a nuclear antigen, all other antigens are expressed on the cell surface (and intracytoplasmatically).

Table 17 Evidence for the origin of HD cell lines

Cell line	*Morphology*	*Cell kinetics*	*Cyto-chemistry*	*Immuno-phenotypes*	*Genotypes*	*Karyotypes*	*Oncogenes*	*Cytokines*	*Functional studies*	*Induction of differentiation*	*Continuous cell line*[1]
CO	incl	L	incl	T	T	incl	incl	T	L, T	T	+
DEV	incl	L	incl	B	B	incl	incl	incl	incl		+
HD-70	incl	L	incl	B	B	incl			incl		+
HDLM-1/2	incl	L	incl	T	T	incl	incl	incl	L, T, H	H	+
HO				T	T						+
HuT-11	incl	Mø	incl			incl			Mø		?
KM-H2	incl	L	incl	B	B	incl	incl	incl	L, H	H	+
L-428	incl	L	incl	incl	B	incl	incl	incl	L, D	incl	+
L-538/540	incl	L	incl	T	T	incl	incl	incl	L		+
L-591		L	incl	B	B	incl		incl	L, B		+
SUP-HD1	incl	L	incl	B	B	incl		T	B, T	T	+
SU/RH-HD-1	incl	Mø	incl	Mø		incl			Mø		?
ZO	incl	L		incl	B	incl			incl		?

[1] ? = cell line not available/no further papers published.
L = lymphoid; T = T-cell; B = B-cell; Mø = monocyte/macrophage; H = histocyte; D = dendritic cells; incl = inconclusive.

similar characteristic features, seem reasonably likely to be authentic H-RS cells. However, definite proof has not and probably cannot be given. They might therefore only operationally be regarded as in-vitro representatives of H-RS cells[56]. Applying strict criteria to the interpretation of the reported data, some doubts have been raised about the identity of some of the described cell lines[46,93,104]: e.g. negativity for CD30 (SUP-HD1, SU/RH-HD-1), unusual clinical description and possibly erroneous diagnosis (DEV). Other cell lines have been described only in one report; as no further data have been published and as these cells have not been made available to other researchers, the immortalization and nature of these cells remain uncertain (HD-70, Hu-T-11, SUP-HD1, SU/RH-HD1, ZO). Also, the H-RS nature of the EBV+ L-591 cells has been questioned as these cells display properties of EBV-transformed LCL[9,100]. In the light of the detection of EBV in uncultured, biopsy H-RS cells[1], others argued that it is justified to consider this cell line as HD-derived[57].

For obvious reasons identification of cultured cell lines as HD-derived is a difficult and subjective multi-step process[10]. Several criteria were defined as evidence for the neoplastic nature of the cultured cells: (i) expansive growth, (ii) clonability on semi-solid medium or in suspension, (iii) tumorigenicity in nude mice, (iv) lack of contact inhibition, (v) aneuploidy and marker chromosomes, (vi) monoclonality of phenotypic expression[20,25,44,47]. However, these definitions are of little help in the distinction of NHL and HD cell lines. Equally, EBV+ LCL in long-term culture might acquire features of neoplastic cells such as expansive growth, clonability, tumorigenicity, aneuploidy and monoclonality[31,43,44].

The relationship of the HD cell lines to the putative malignant H-RS cells in HD tissues in-vivo remains unestablished[52]. Similar immunophenotypic features and, in some instances, similar antigen receptor gene rearrangements have been ascribed to native and in-vitro H-RS cells[5,10,25,52]. H-RS cells in culture are no longer in their natural environment exposed to many interactional phenomena[11]. The necessary selection of one clone as the basis for the establishment and expansion of the cells carries the risk of generating a cell line from an atypical clonable cell, and consequently, the possibility of obtaining a culture with atypical phenotypic attributes. The method of culturing might selectively enhance the emergence of different cell lines with different features (thus explaining the pronounced heterogeneity among the HD cell lines).

The cultured cells represent a small population of tumor cells that can adapt to an artificial milieu. Furthermore, certain clinical conditions favor the continuous culturing of H-RS cells[5]: advanced clinical disease (8 out of 12 cell lines), nodular sclerosing histological subtype (11 out of 13), and cells from liquid body systems (9 out of 13). Patients with advanced disease had received chemo- and radiotherapy which might have caused genetic or epigenetic changes in the H-RS cells enabling autonomous cell growth. Possible explanations for the apparent advantage of pleural fluid over lymph node and spleen tissue are the adaptation of the H-RS cells to grow in suspension and the absence of reactive elements such as macrophages and eosinophilic granulocytes[35]. In several hundred attempts predominantly cell lines from NS type HD were established[61]. Biopsy H-RS cells from patients with HD of the NS type have many characteristics of lymphoid cells. The artificial in-vitro system has growth conditions particularly advantageous for lymphocytes[61]. Thus, the predominance of NSHD-derived cell lines is likely to be another culture bias[10]. HD cell lines have not demonstrated many consistent properties and there is no proof, as yet, of a common progenitor cell giving rise to the variety of HD cell lines. There is a striking heterogeneity of morphological variations among the cell lines, but also within the population of each cell line. However, despite the morphological diversity among individual cells, the monoclonality of the populations was shown by cytogenetic markers and gene rearrangement analysis. Morphological and cytochemical examinations do not contribute any clues as to the likely origin of the cells.

Cell kinetic studies established that the clonogenic cell in the HD cell lines is the mononucleated cell from which the polynucleated cells develop by endomitosis and disturbed cytokinesis[49,51]. The giant, polynucleated cells are capable of DNA synthesis and nuclear division, but do not multiply and survive not longer than several weeks before undergoing senescence[49–51].

The HD cell lines lack immunological markers or composite immunophenotypes that would define them unequivocally as lymphoid or myeloid cells. The absence of definitive immunological lineage markers demonstrates again that these cells cannot easily be correlated to a known cell type. No normal cell in the hematopoietic system shares these unusual immunoprofiles[54]. Some cell lines express a mixed lineage phenotype[52]. The detection of various receptor gene rearrangements in the HD cell lines promotes the

concept of a lymphoid derivation[7]. The common denominator in several cell lines is the presence of rearrangements in the Ig or TCR genes which are inadequate for the formation of a functional antigen receptor[55]. With few exceptions, all these rearrangements are nonproductive. Overall, genotypic characterization of HD cell lines cannot provide a definitive answer concerning the cellular origin. The cytogenetic analysis of the HD cell lines did not reveal the existence of a typical and specific chromosomal marker[67,104]. Several cell lines are characterized by great chromosomal instability[5,25]. The possibility cannot be excluded that some or all of the described chromosomal alterations were caused by radiation or chemical mutagenesis during treatment of the patients[67]. Oncogene expression represents another set of features associated with HD cell line heterogeneity. Normal and aberrant transcripts of proto-oncogenes that are not found in untransformed hematopoietic cells were detected[63]. The heterogeneity might reflect variable differentiation stages, deregulation or activation of the genes[5]. It cannot be excluded that the cell lines acquired the expression of some oncogenes during the course of culture[63]. Expression and production/secretion of cytokines and their receptors are among the most distinctive features of HD cell lines. The characteristic histology of HD might be caused by the production and release of cytokines by H-RS cells[1,79,80]. Cytokines produced by H-RS cells may act in an autocrine mode or may be secreted in a paracrine or endocrine fashion[74]. Furthermore, the abnormal or unbalanced secretion of such factors could be responsible for the systemic B symptoms in HD like weight loss, fever and night sweats[56,80]. Clearly, in-vitro studies may not reflect the situation in-vivo[81] and the production of cytokines may be acquired after adaptation in culture[76]. Some cells might have the ability to aberrantly express specific factors and their receptors[86].

The failure of the cultured H-RS cells to respond to exogenous cytokines suggests that, during the course of neoplastic transformation, of disease progression, or of in-vitro culturing the cells lose their dependence on cytokines[77]. The biological factors are not useful for clarifying the histogenetic H-RS cell derivation since a variety of normal cell types release one or more of these biomodulators[61]. Nevertheless, these observations provide a starting point for the analysis of micro-environmental stimuli accounting for the particular histological picture.

Functional studies could not be performed with the native H-RS cells. The use of in-vitro cells allowed for the investigation of the functional properties of these cells[3]. The mixture of results obtained does not add much evidence for derivation from a defined lineage, but rather helps to exclude certain cell types[10]. Induction of differentiation studies showed that treatment with modulating agents triggered alterations compatible with histiocyte-like cells. It is important to realize that activation with TPA is non-specific and that certain tumor cells may differentiate along unexpected pathways[13].

The data in all areas of investigation have clearly demonstrated the heterogeneity among the HD cell lines preventing a conclusive definition of the nature of the in-vivo H-RS cell. Some authors interpret the results as evidence for a lymphoid or a monocyte/histiocyte derivation[5,7,58]. Others suggested an undifferentiated stem cell or a natural hybridoma[12,40]. Rather than resolve the question on the origin of H-RS cells, the established HD cell lines confirm the diversity of features seen in in-vivo H-RS cells.

FUTURE DIRECTIONS

Both cellular and molecular approaches will be required to fully understand cell lineage commitment and phenotype of H-RS cells. Knowledge of the biology of these curious cells will likely prove relevant to a better understanding of the disease. HD cell lines were useful for the last decade as targets of intensive study on HD-related phenomena. The cell lines have provided new vistas concerning the origin and function of HD tumor cells. The analysis of cytokine biology and its unique interweaving of autocrine, paracrine and endocrine patterns of cell-to-cell interaction has generated important biological and possibly diagnostic information. This knowledge will be useful for the purposes of clinical management.

The caveats with regard to cell lines are that these cells do not necessarily represent in-vivo biology due to selection of cells adaptable to artificial in-vitro conditions and due to the possible acquisition or loss of certain properties in culture. Attempts should be undertaken to establish more HD cell lines from histological subtypes other than nodular sclerosis and from lymph node or spleen specimens. Interest must also go back to primary tumor tissue and hypotheses based on investigations with HD cell lines must be verified on biopsy H-RS cells.

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